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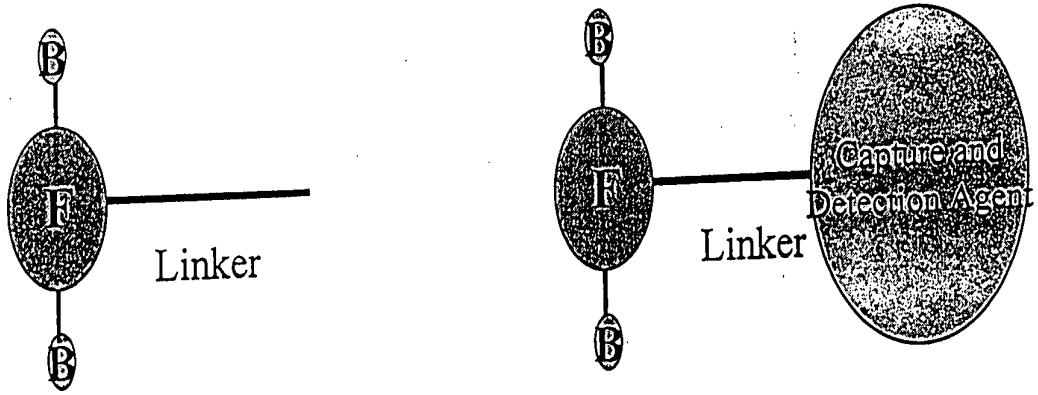
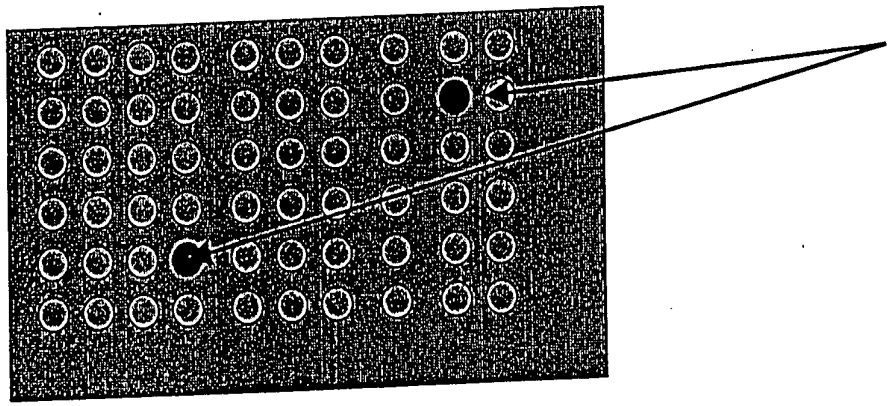
High-throughput Target ID



Library of Bioactive Compounds

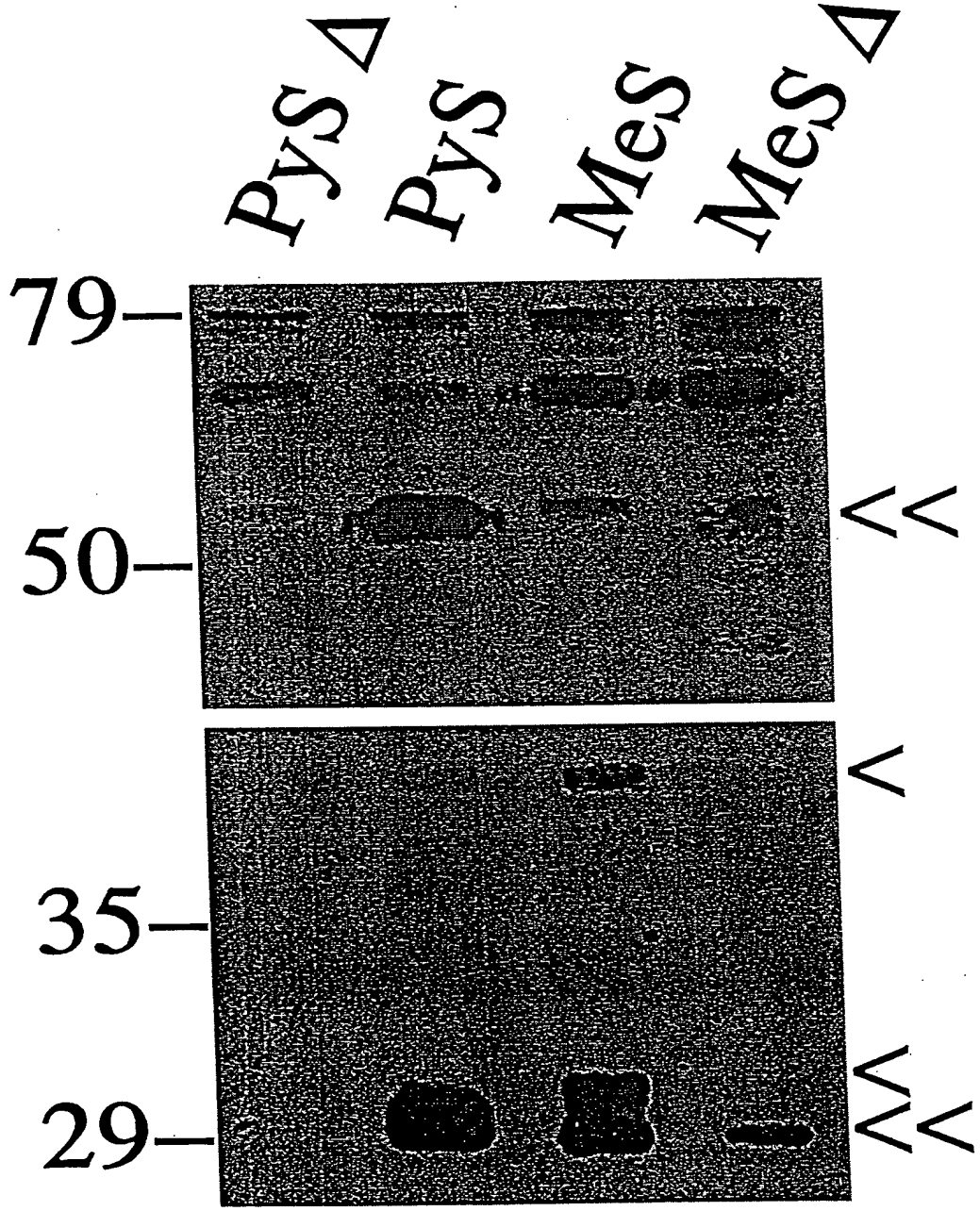
Library of Target ID Compounds



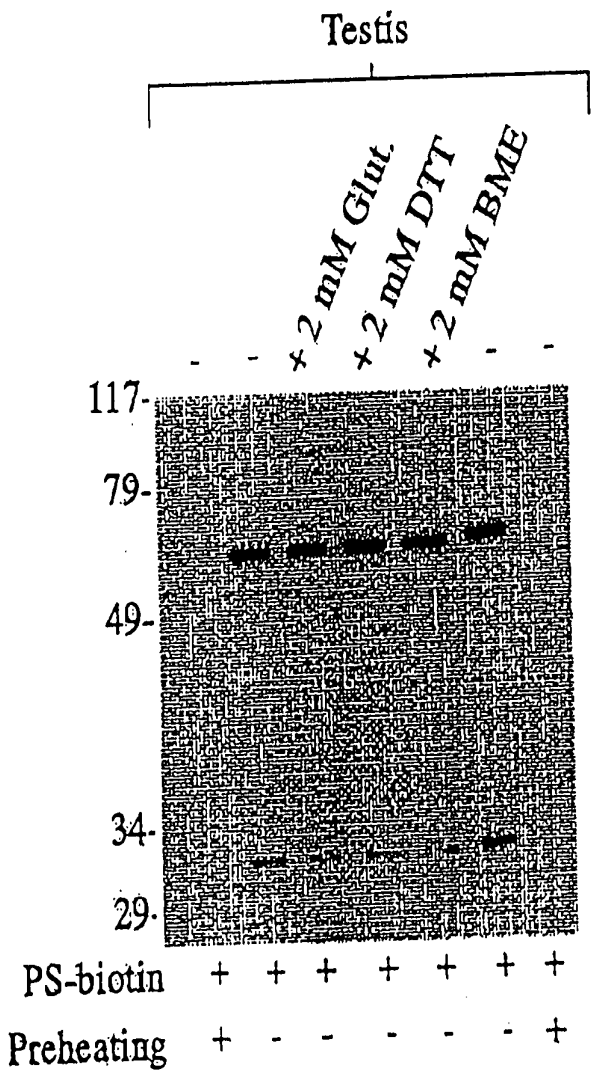
Use corresponding activity-based probe to identify the biological target

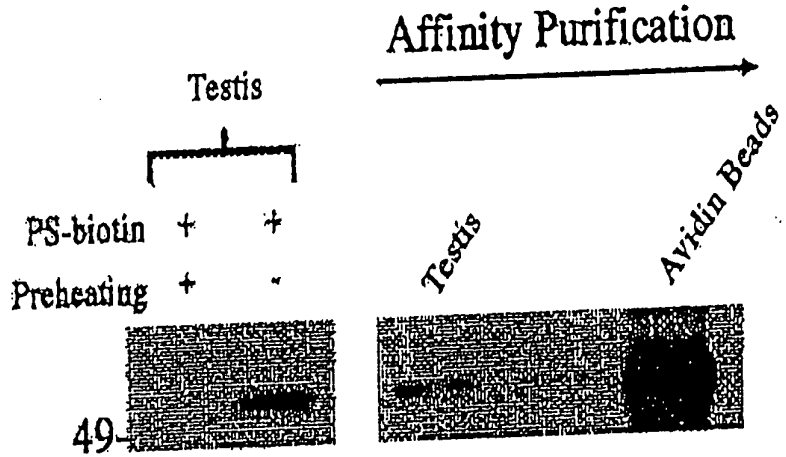
FIGURE 1

FIGURE 2



Non-Directed Tagged Library of Sulfonates Identifies Probe for ADH Superfamily of Enzymes

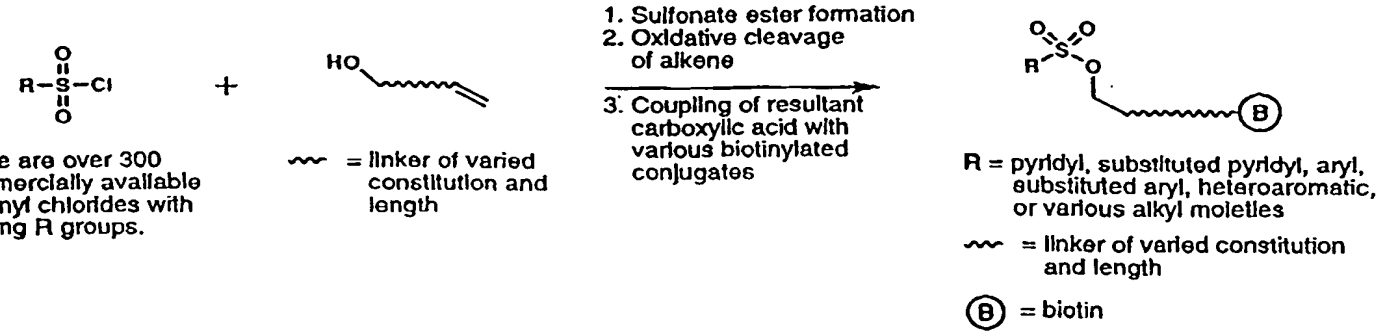




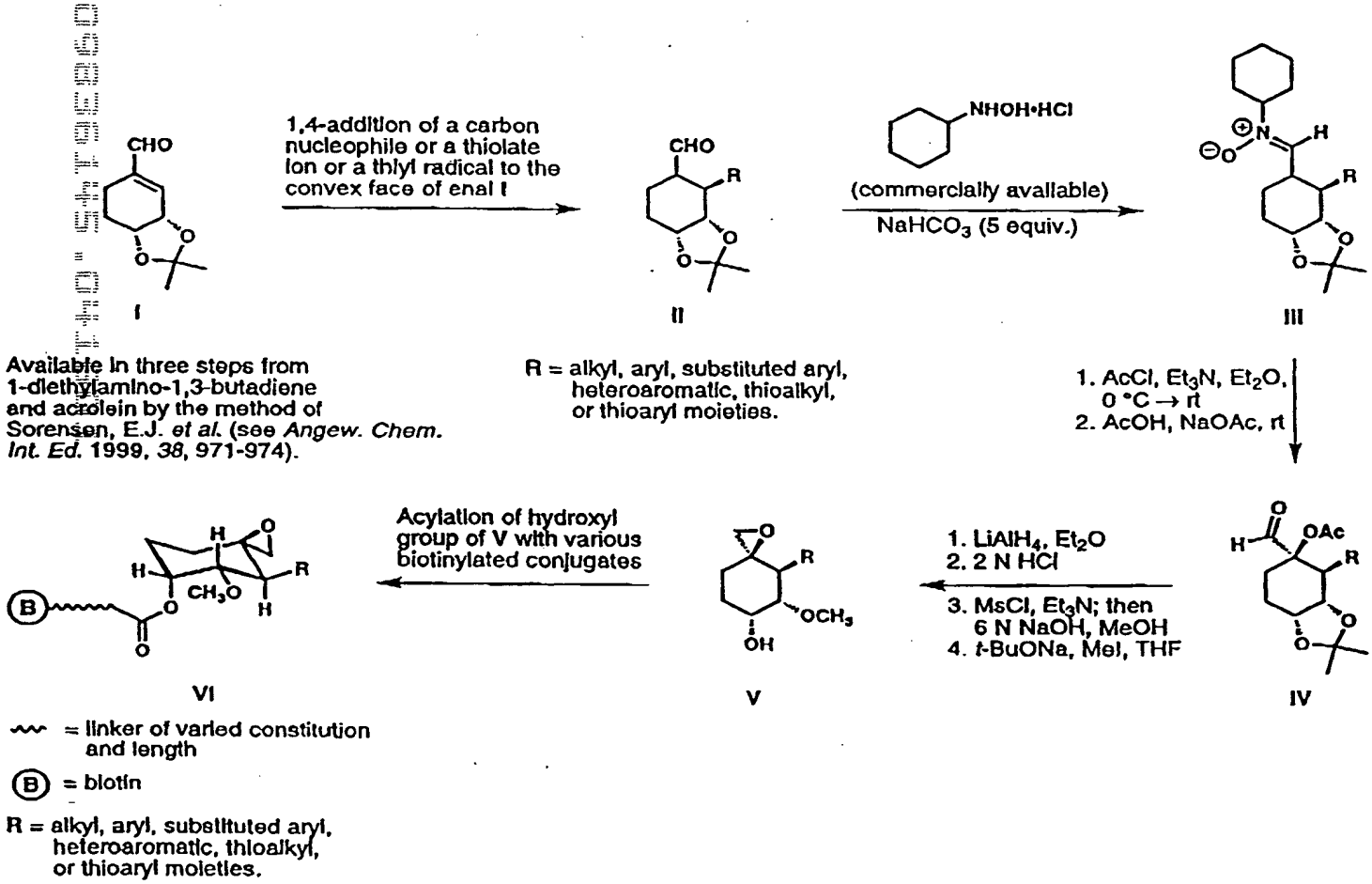
- MALDI mapping identifies tagged protein as aldehyde dehydrogenase (ADH, cytosolic class II)
- 28 ADHs in fly genome
  - Involved in retinoic acid biosynthesis and catabolism of alcohol and chemotherapeutic agents

FIGURE 3

FIGURE 4



Scheme 1. A pathway for syntheses of various biotinylated sulfonate esters for use in activity-based proteomics studies.



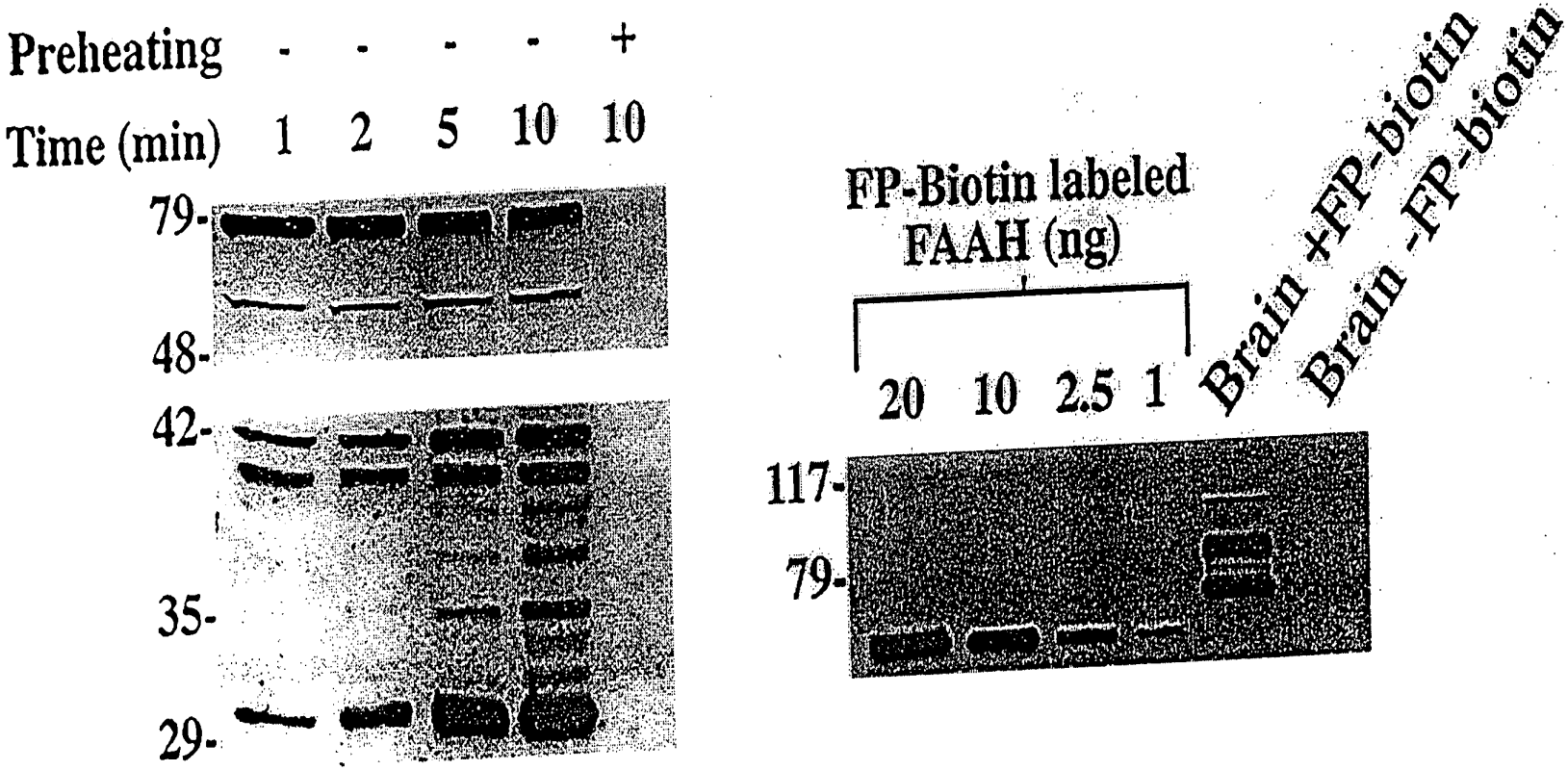
Scheme 2. A strat gy for converg nt, stereocontrolled syntheses of conformationally well-defined spiroepoxides of type VI. Literature precedent for I → II → III → IV → V can be found in Sorensen, E.J. *et al. Angew. Chem. Int. Ed.* 1999, *38*, 971-974. Compounds of type VI are analogs of the metalloprotease (MetAp-2) inhibitor fumagillin and will be employed as covalent affinity agents in activity-bas d proteomics studies.

FP-Biotin: a kinetic reporter of SH Activity

The rates at which the majority of SHs react with FP-biotin can be experimentally followed

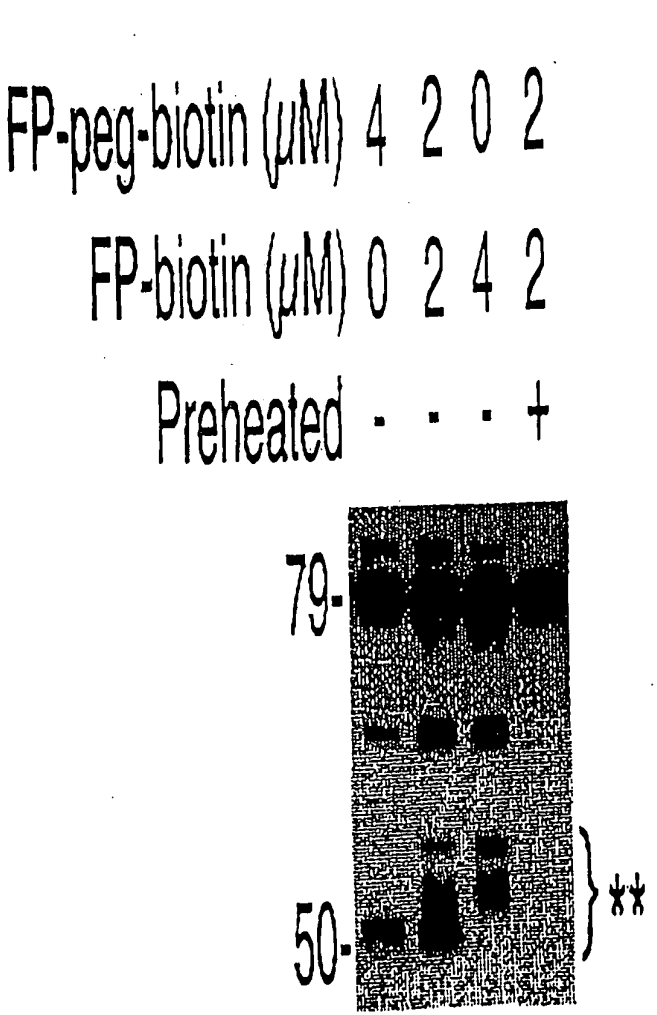
FP-biotin readily detects low femtomole quantities of SHs directly in complex cell/tissue proteomes

FIGURE 5



Utility of Multiplexed probes in identifying Serine Hydrolases

FIGURE 6



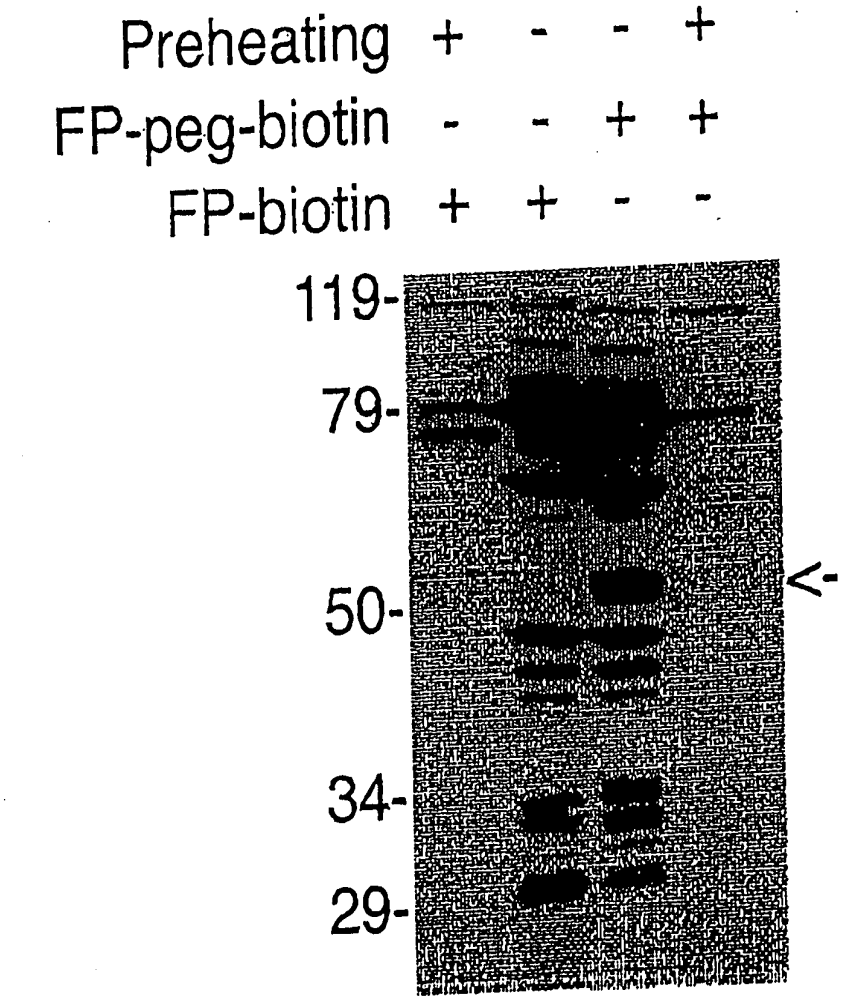


FIGURE 7

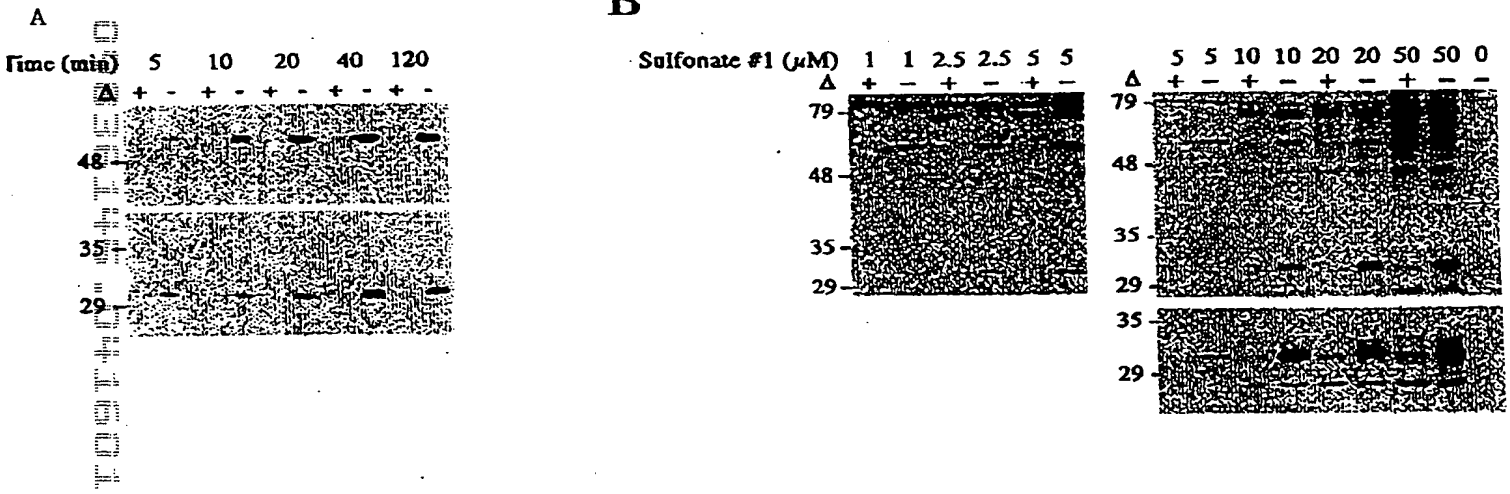
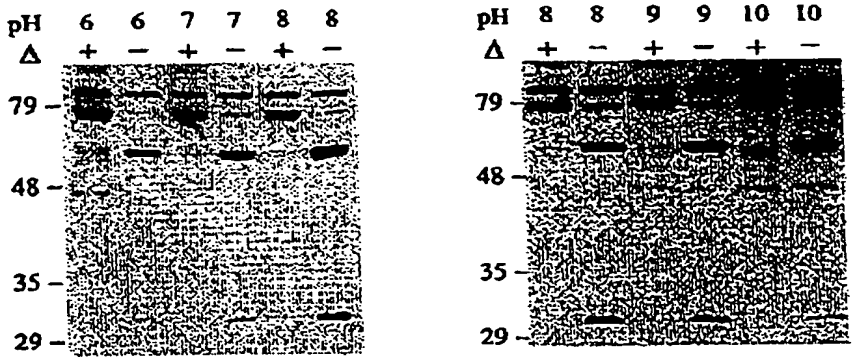

A
Time (min) 5 10 20 40 120
Δ + - + - + - + - + -
48
35
29
B
Sulfonate #1 (μM) 1 1 2.5 2.5 5 5
Δ + - + - + -
79
48
35
29
5 5 10 10 20 20 50 50 0
Δ + - + - + - + - -
79
48
35
29
35
29

FIGURE 7

C


pH 6 6 7 7 8 8
Δ + − + − + −
79
48
35
29
pH 8 8 9 9 10 10
Δ + − + − + −
79
48
35
29

D

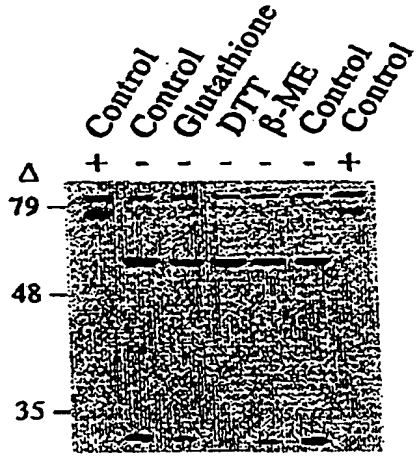

Control
Control
Glutathione
DTT
β-ME
Control
Control
Δ + - - - - - +
79
48
35

FIGURE 8

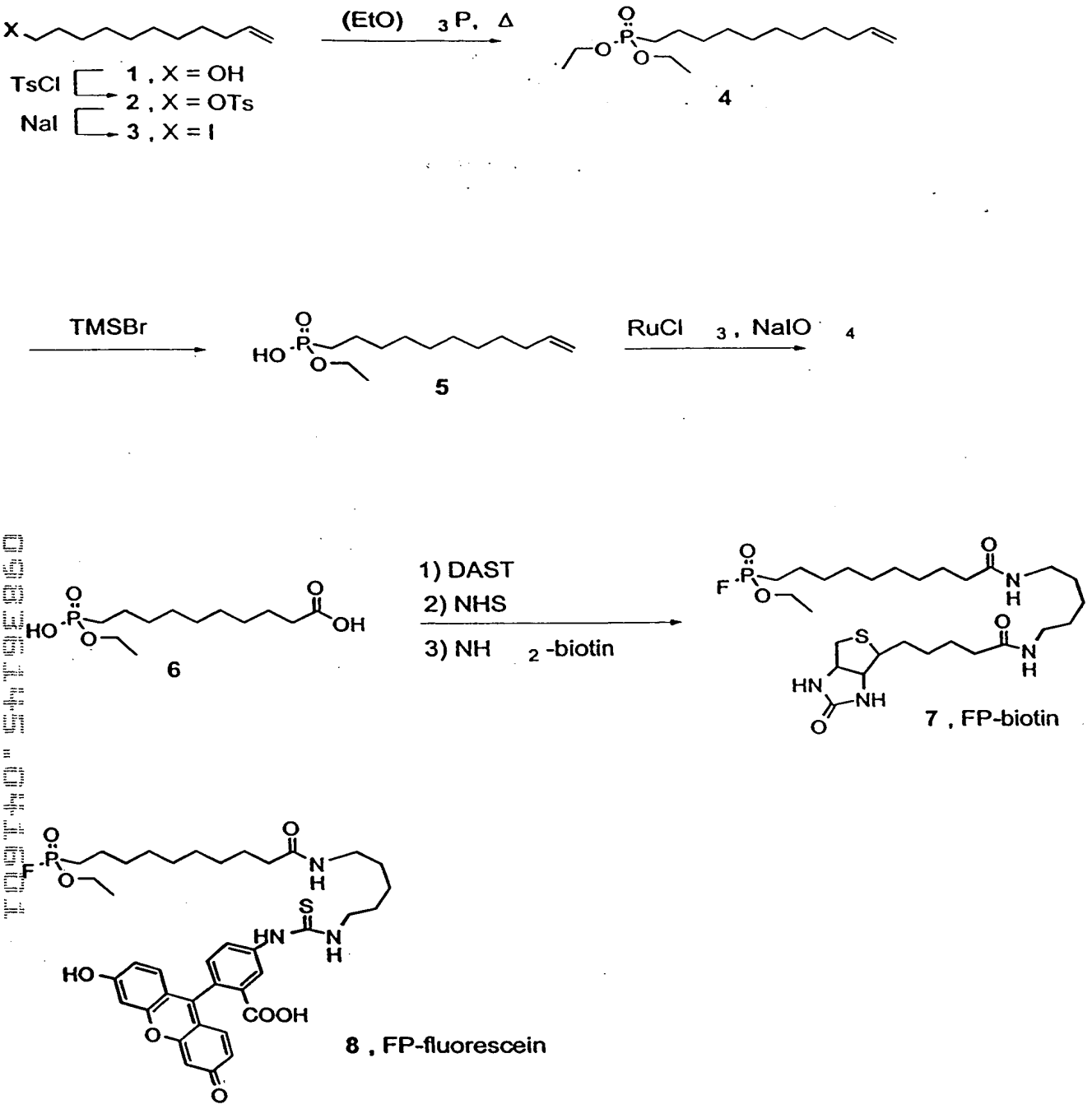

X
TsCl
NaI
1 , X = OH
2 , X = OTs
3 , X = I
(EtO) 3 P, Δ
4
TMSBr
5
RuCl 3 , NaIO 4
6
1) DAST
2) NHS
3) NH 2 -biotin
7 , FP-biotin
8 , FP-fluorescein

FIGURE 9

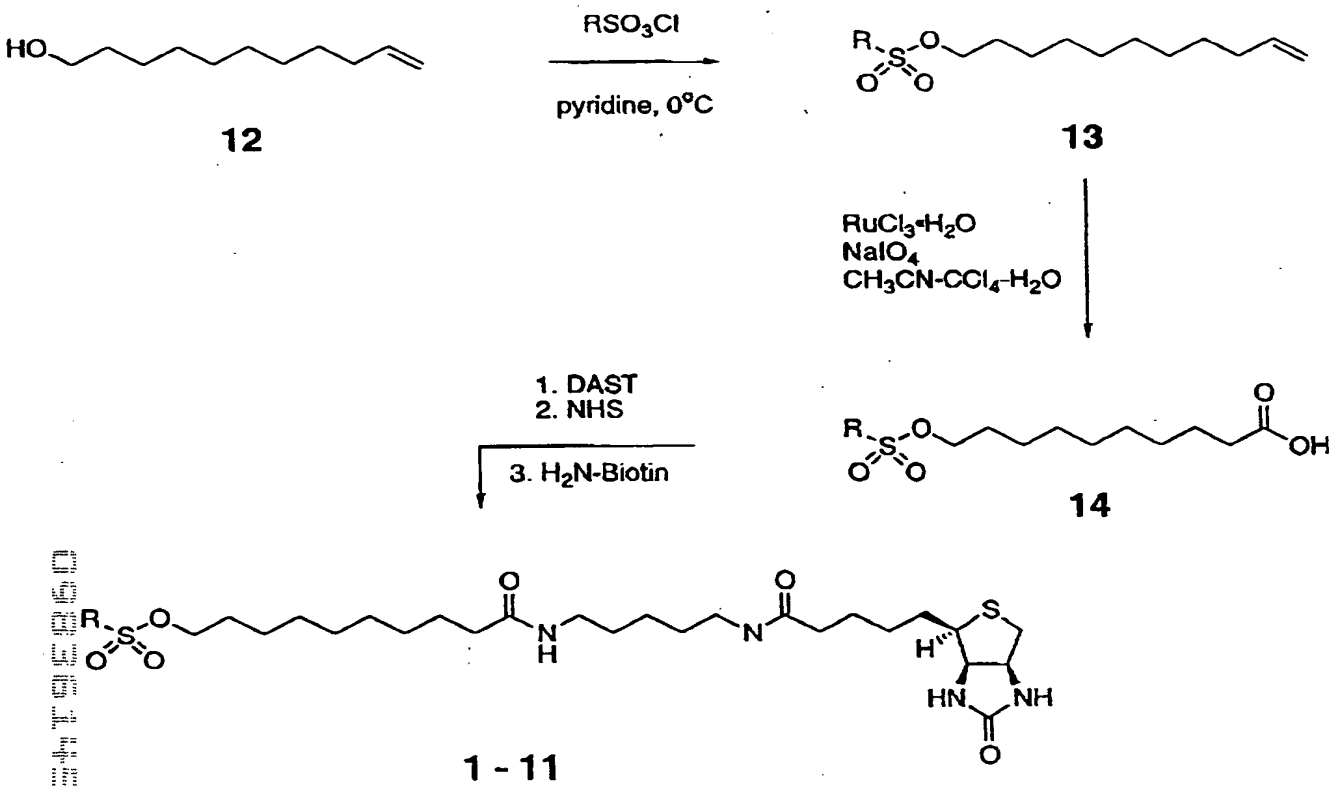
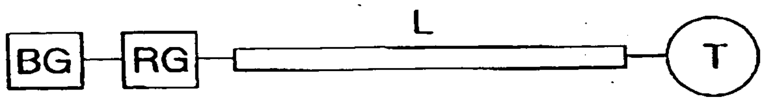


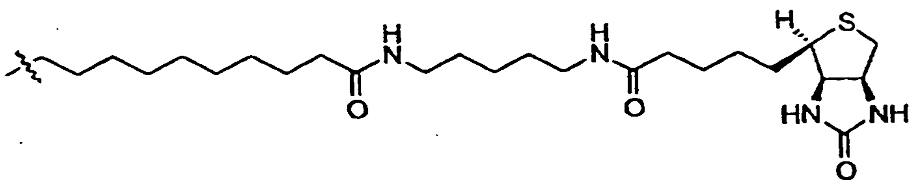
FIGURE 10

A.

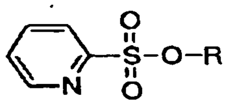


B.

R =



1



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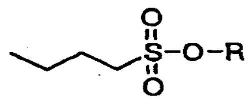

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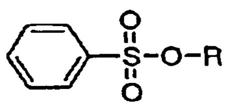

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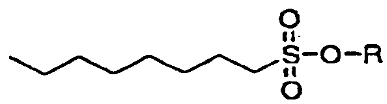

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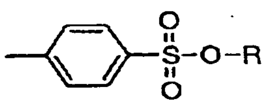

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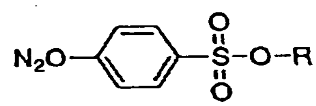

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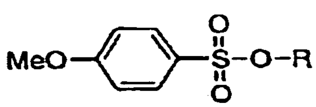

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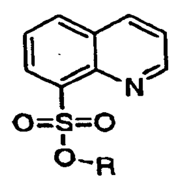

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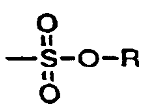

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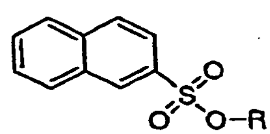

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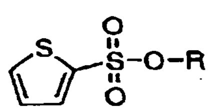


FIGURE 11

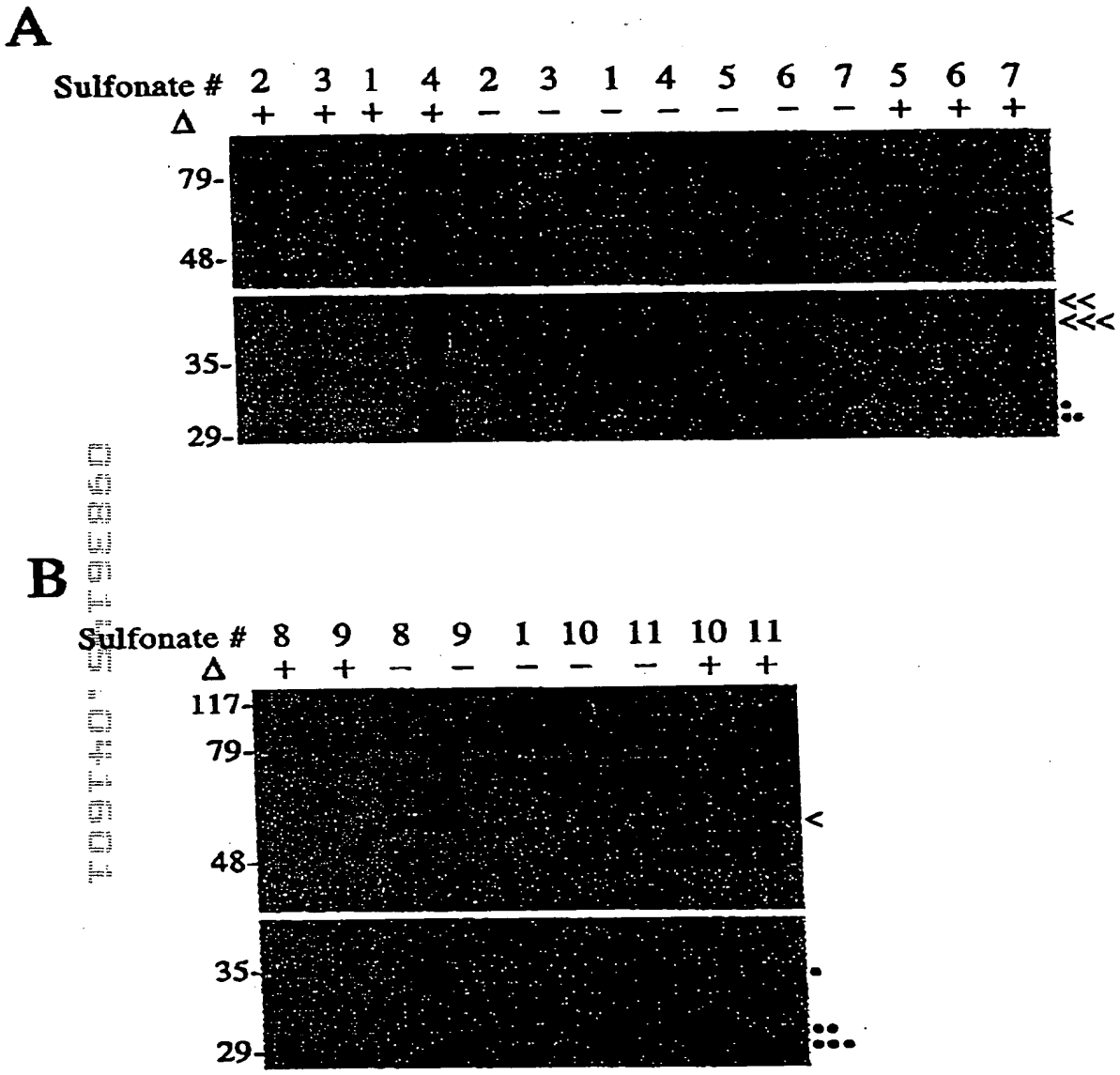

A
Sulfonate # 2 3 1 4 2 3 1 4 5 6 7 5 6 7
Δ + + + + − − − − − − − + + +
79-
48-
35-
29-
<
<<
<<<
B
Sulfonate # 8 9 8 9 1 10 11 10 11
Δ + + − − − − − + +
117-
79-
48-
35-
29-
<

FIGURE 12

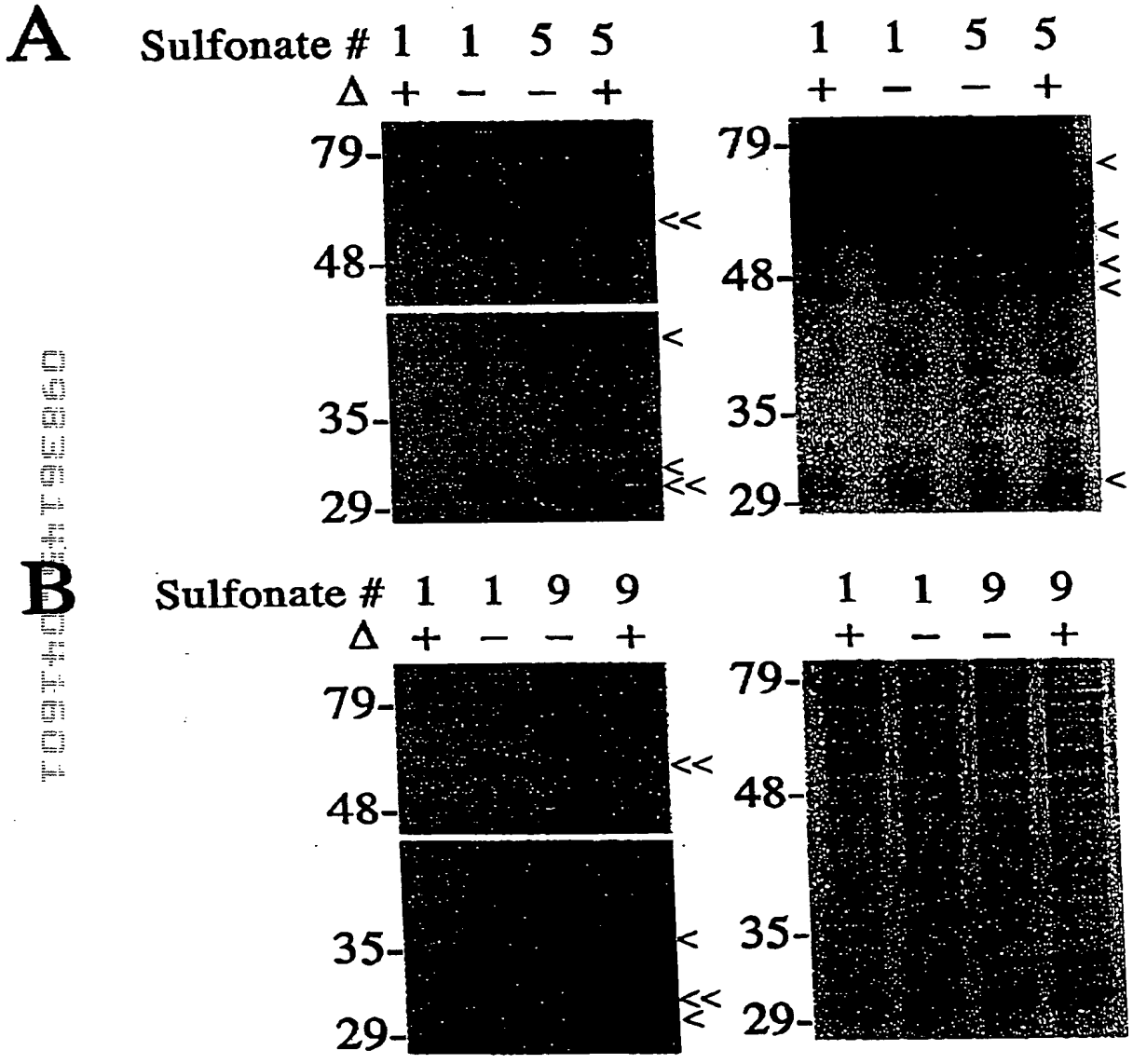
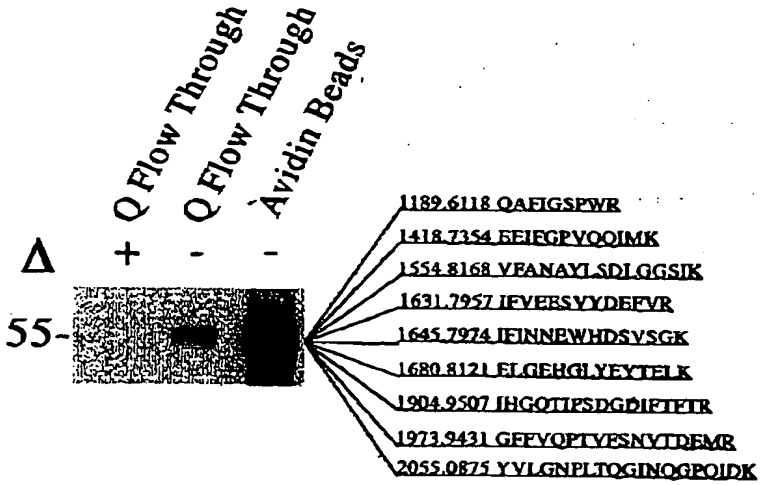
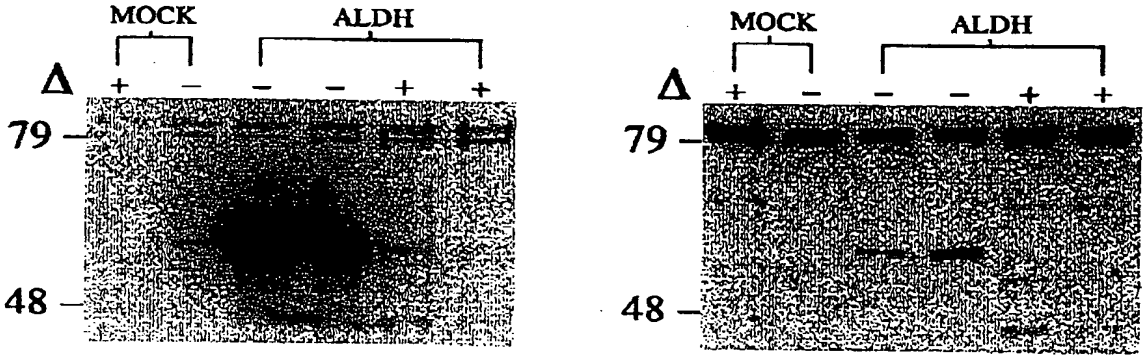

A
Sulfonate # 1 1 5 5
Δ + − − +
79-
48-
35-
29-
1 1 5 5
+ − − +
79-
48-
35-
29-
B
Sulfonate # 1 1 9 9
Δ + − − +
79-
48-
35-
29-
1 1 9 9
+ − − +
79-
48-
35-
29-

FIGURE 13

A



B



C

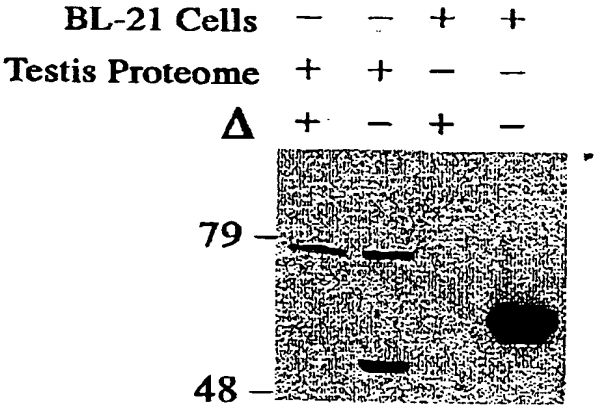
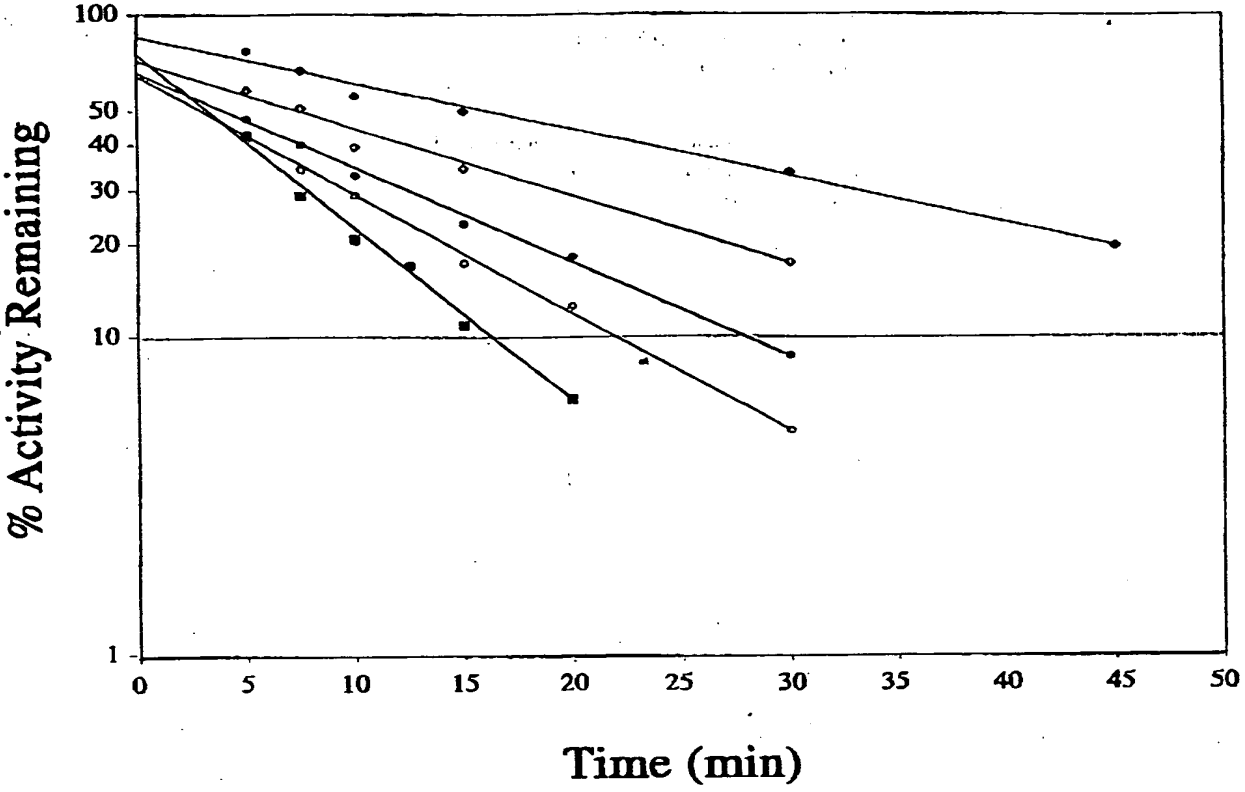


FIGURE 14

A


100
50
40
30
20
10
1
% Activity Remaining
0
5
10
15
20
25
30
35
40
45
50
Time (min)

B

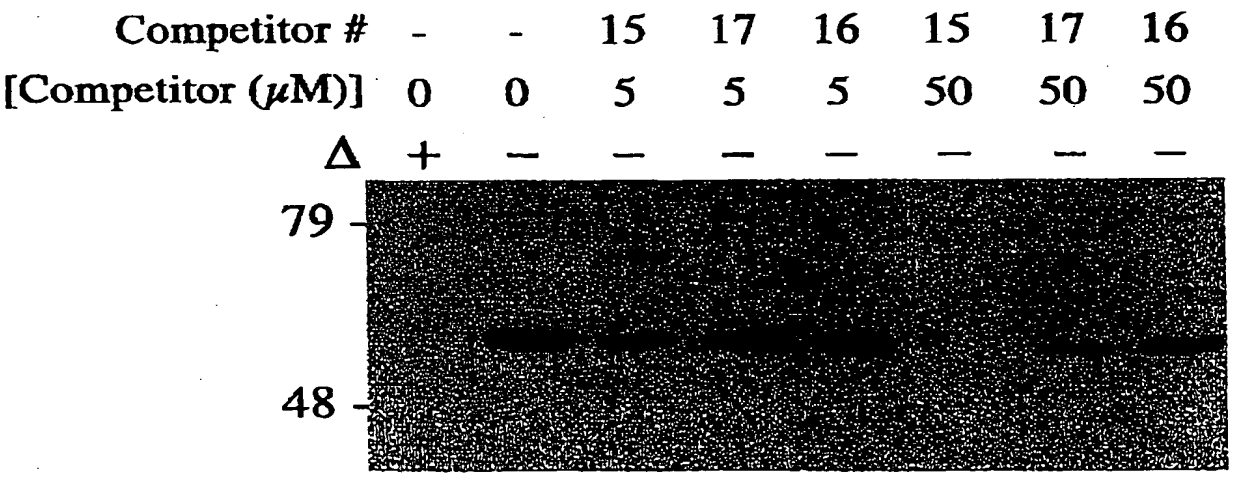

Competitor # - - 15 17 16 15 17 16
[Competitor (μM)] 0 0 5 5 5 50 50 50
Δ + — — — — — — —
79
48

FIGURE 15

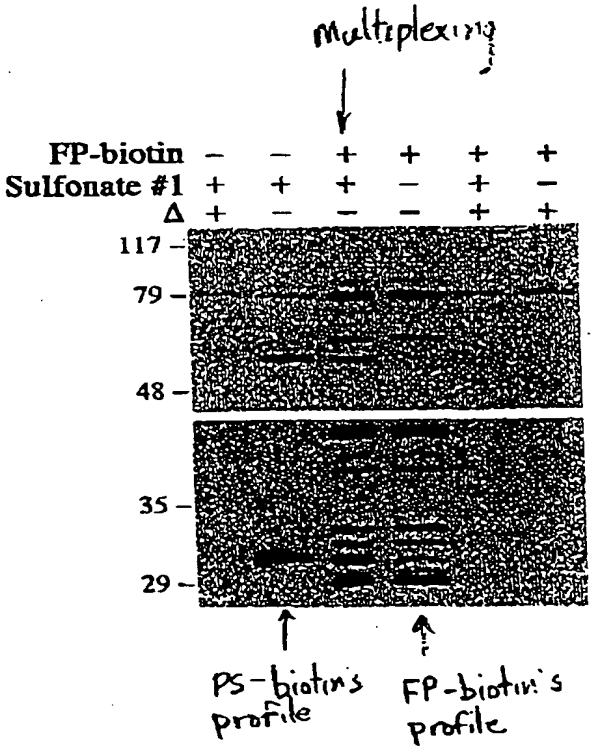

Multiplexing
FP-biotin – – + + + +
Sulfonate #1 + + + – + –
Δ + – – – + +
117 –
79 –
48 –
35 –
29 –
PS-biotin's profile
FP-biotin's profile

FIGURE 16

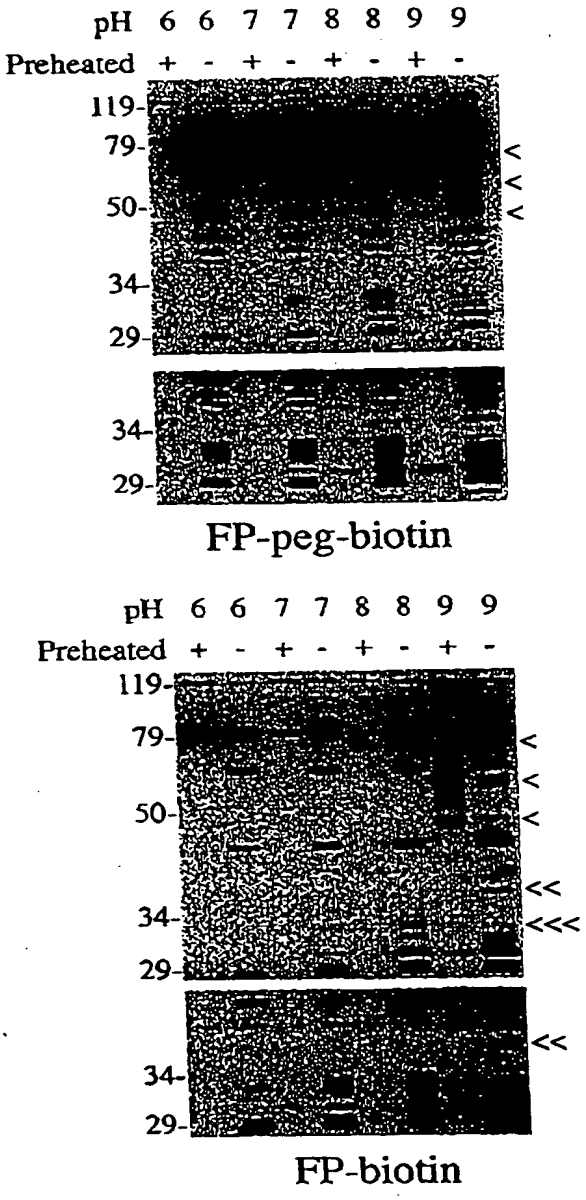

pH 6 6 7 7 8 8 9 9
Preheated + - + - + - + -
119-
79-
50-
34-
29-
<
<
<
34-
29-
FP-peg-biotin
pH 6 6 7 7 8 8 9 9
Preheated + - + - + - + -
119-
79-
50-
34-
29-
<
<
<
<<
<<<
34-
29-
<<
FP-biotin

FIGURE 17

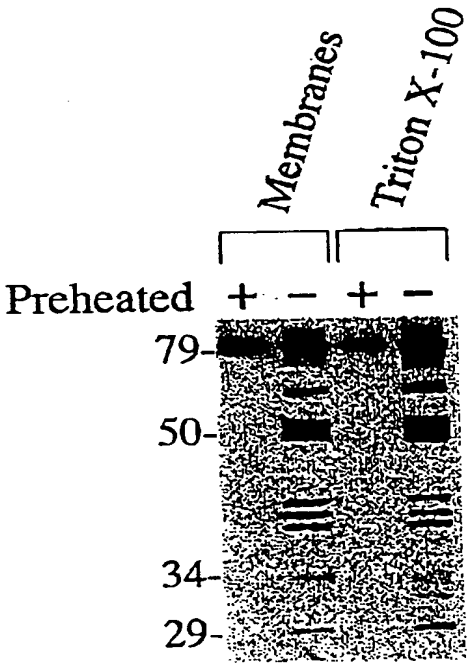


FIGURE 18

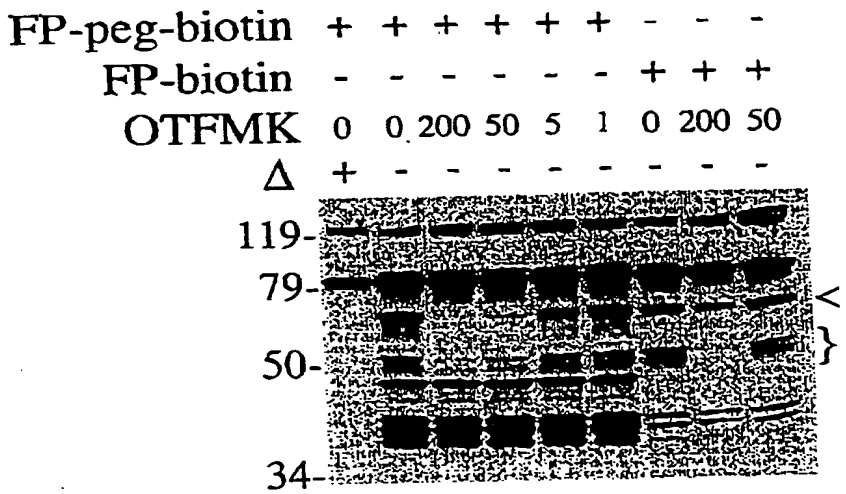


FIGURE 19

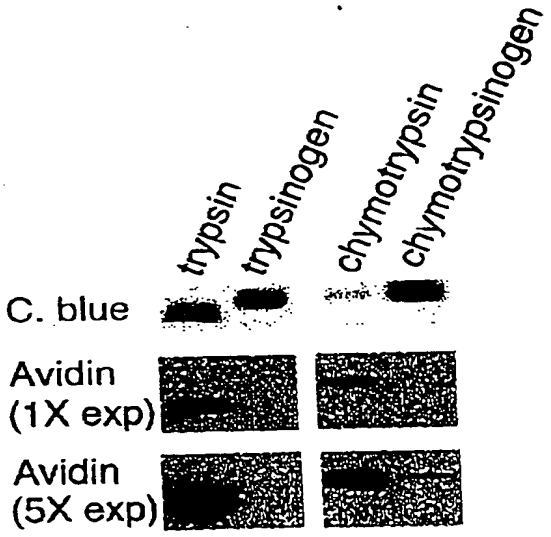
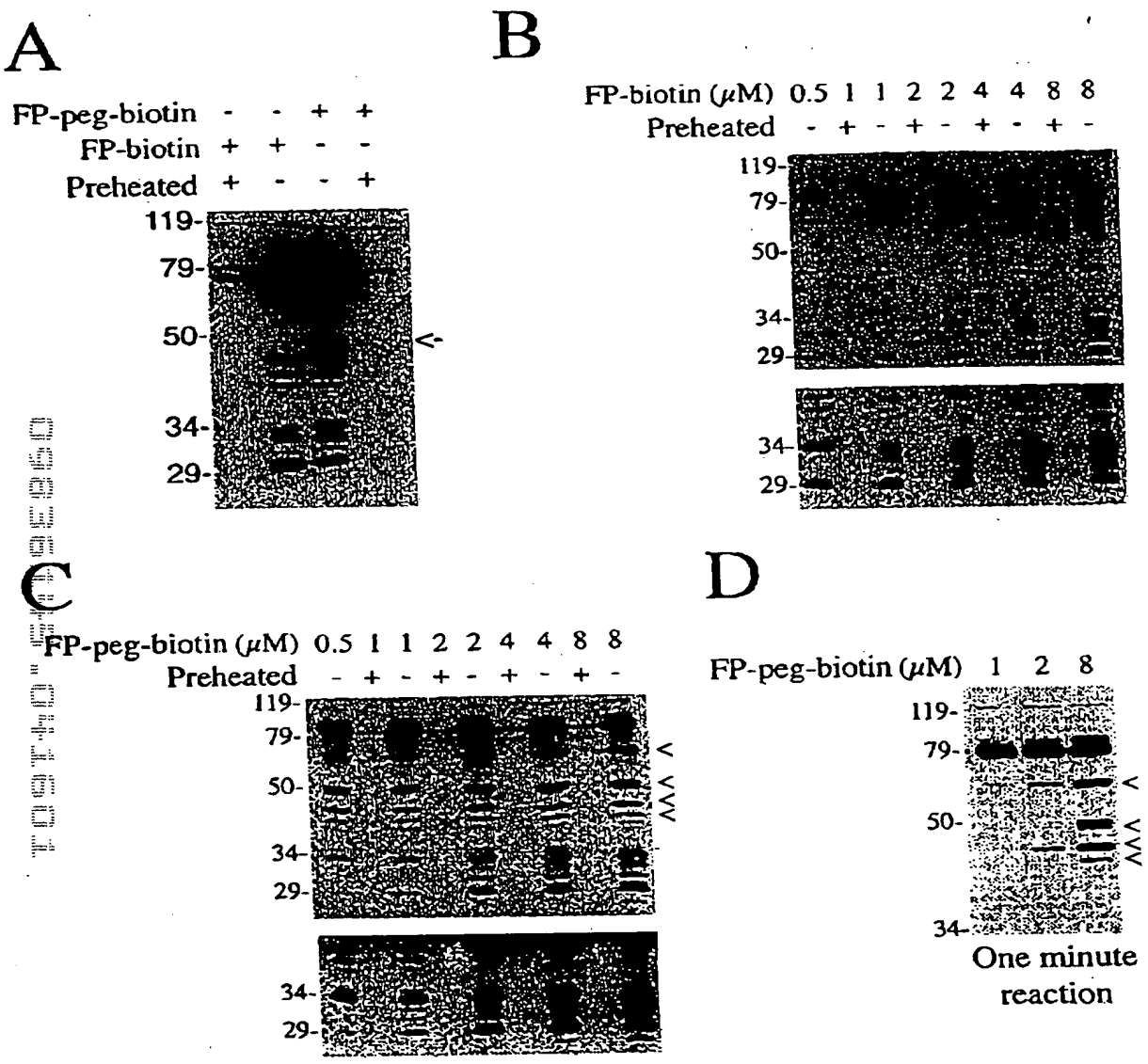


FIGURE 20


A
FP-peg-biotin - - + +
FP-biotin + + - -
Preheated + - - +
119-
79-
50-
34-
29-
<-
B
FP-biotin (μM) 0.5 1 1 2 2 4 4 8 8
Preheated - + - + - + - + -
119-
79-
50-
34-
29-
34-
29-
C
FP-peg-biotin (μM) 0.5 1 1 2 2 4 4 8 8
Preheated - + - + - + - + -
119-
79-
50-
34-
29-
<
<<<
34-
29-
D
FP-peg-biotin (μM) 1 2 8
119-
79-
50-
34-
<
<<<
One minute reaction

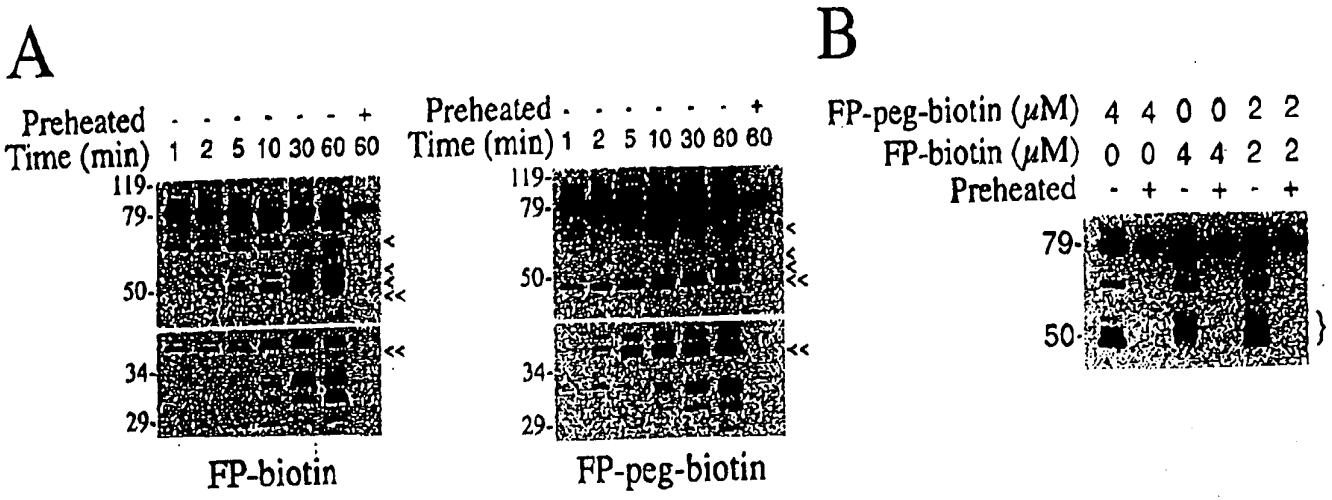


FIGURE 21

FIGURE 22

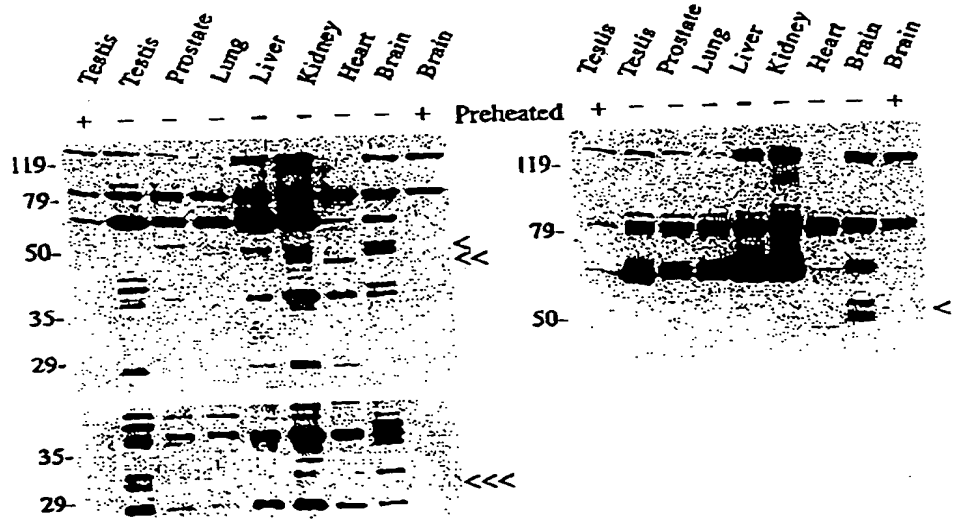
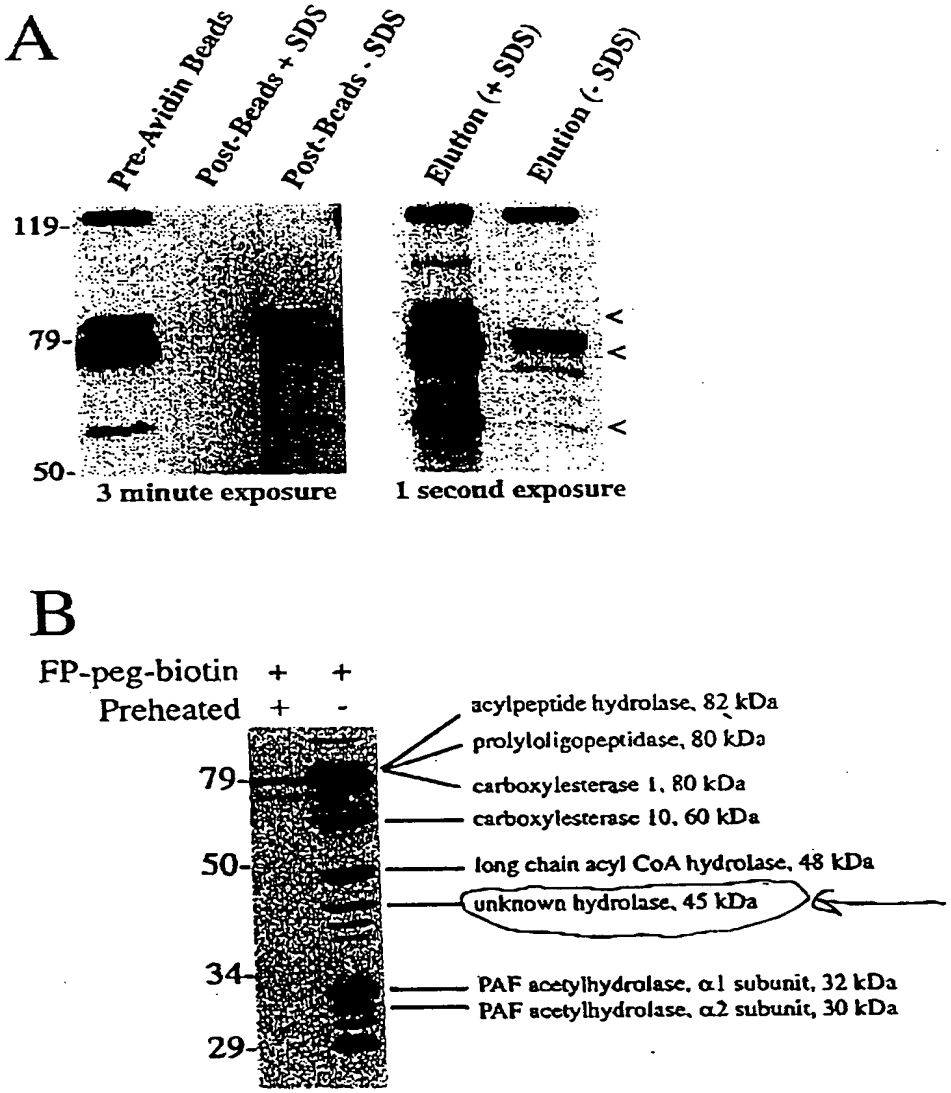

Testis
Testis
Prostate
Lung
Liver
Kidney
Heart
Brain
Brain
Preheated
119-
79-
50-
35-
29-
<<<
<

FIGURE 23


A
Pre-Avidin Beads
Post-Beads + SDS
Post-Beads - SDS
Elution (+ SDS)
Elution (- SDS)
119-
79-
50-
3 minute exposure
1 second exposure
B
FP-peg-biotin + +
Preheated + -
79-
50-
34-
29-
acylpeptide hydrolase, 82 kDa
prolyloligopeptidase, 80 kDa
carboxylesterase 1, 80 kDa
carboxylesterase 10, 60 kDa
long chain acyl CoA hydrolase, 48 kDa
unknown hydrolase, 45 kDa
PAF acetylhydrolase, α1 subunit, 32 kDa
PAF acetylhydrolase, α2 subunit, 30 kDa